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FbsC, a Novel Fibrinogen-binding Protein, Promotes *Streptococcus agalactiae*-Host Cell Interactions*

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Background: *Streptococcus agalactiae* (GBS) must bind to fibrinogen to cross host barriers and cause disease.
Results: A novel fibrinogen-binding protein of GBS, named FbsC, was shown to be required for efficient invasion of human cells.
Conclusion: GBS utilizes FbsC to adhere to fibrinogen on human cells and invade them.
Significance: Blocking the function of FbsC may be useful to prevent or treat infections by GBS.

Streptococcus agalactiae **(group B *Streptococcus* or GBS) is a common cause of invasive infections in newborn infants and adults. The ability of GBS to bind human fibrinogen is of crucial importance in promoting colonization and invasion of host barriers. We characterized here a novel fibrinogen-binding protein of GBS, designated FbsC (Gbs0791), which is encoded by the prototype GBS strain NEM316. FbsC, which bears two bacterial immunoglobulin-like tandem repeat domains and a C-terminal cell wall-anchoring motif (LP*X*TG), was found to be covalently linked to the cell wall by the housekeeping sortase A. Studies using recombinant FbsC indicated that it binds fibrinogen in a dose-dependent and saturable manner, and with moderate affinity. Expression of FbsC was detected in all clinical GBS isolates, except those belonging to the hypervirulent lineage ST17. Deletion of *fbsC* decreases NEM316 abilities to adhere to and invade human epithelial and endothelial cells, and to form biofilm *in vitro*. Notably, bacterial adhesion to fibrinogen and fibrinogen binding to bacterial cells were abolished following *fbsC* deletion in NEM316. Moreover, the virulence of the *fbsC* deletion mutant and its ability to colonize the brain were impaired in murine models of infection. Finally, immunization with recombinant FbsC significantly protected mice from lethal GBS challenge. In conclusion, FbsC is a novel fibrinogen-binding protein expressed by most GBS isolates that functions as a virulence factor by promoting invasion of epithelial and endothelial barriers. In addition, the protein has significant immunoprotective activity and may be a useful component of an anti-GBS vaccine.**

Infections by *Streptococcus agalactiae* (group B *Streptococcus*, GBS)[4] are a major health problem worldwide (1, 2). This Gram-positive bacterium persists as the main cause of life-threatening conditions in the neonate, including pneumonia, sepsis, and meningitis. In addition, the incidence of infections in adults with underlying chronic disease and in elderly people has been steadily increasing in recent years (3). GBS is part of the normal flora of the intestine, which represents the main reservoir of this organism, and is also found in the vagina of 15–30% of healthy women (4). The ability of these bacteria to adhere to mucosal epithelial cells, particularly to those of the respiratory and intestinal tracts, is a crucial determinant of colonization and infection. Moreover, translocation across epithelial and endothelial barriers is a necessary step for these bacteria to reach the bloodstream and eventually spread to target organs, including the meninges and the central nervous system (5, 6). Both colonization and invasion of host barriers by GBS are related to their ability to bind to extracellular matrix proteins, particularly to human fibrinogen (7–9). The interaction of GBS with human fibrinogen has been reported by several authors (10, 11). Because it is present in plasma, tissues, and on the surface of host cells (12), fibrinogen acts as a molecular bridge between GBS and human tissues and can participate in a number of pathogenic processes, including colonization of mucosal surfaces, biofilm formation, invasion of epithelial and endothelial cells, interference with phagocytosis and thrombus formation (9). It has been well established that strains causing severe invasive infections display stronger binding to fibrinogen than colonizing strains (13).

GBS interactions with fibrinogen were initially associated with the expression of the cell wall-anchored LP*X*TG protein FbsA and of the secreted protein FbsB, two structurally unrelated proteins that are both capable of binding fibrinogen *in vitro* (14–16). FbsA might be involved in adhesion to epithelial cells (7), but not in cell invasion, a process for which FbsB is

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[4] The abbreviations used are: GBS, group B *Streptococcus*; SrtA, sortase A; CC, clonal complex.

TABLE 1
GBS strains

The abbreviations used include: aTc, anhydrotetracycline; pTCV_TetO, "empty" vector with an aTc-inducible promoter; pTCV_TetO_*fbsC*, vector with the *fbsC* gene under the control of the aTc-inducible promoter.

ID strains	Strains	Features
NEM316	GBS WT	Clinical isolate, serotype III[a]
NEM2511	GBS *SrtA**	NEM316 with an inactive sortase A enzyme[b]
NEM3296	GBS Δ*fbsC*	*fbsC* deletion in NEM316
NEM3788	GBS WT + pTCV_TetO	NEM316 with the empty vector
NEM3790	GBS WT + pTCV_TetO_*fbsC*	NEM316 with aTc-inducible *fbsC* vector
NEM3792	GBS Δ*fbsC* + pTCV_TetO	NEM3296 with the empty vector
NEM3794	GBS Δ*fbsC* + pTCV_TetO_*fbsC*	NEM3296 with aTc-inducible *fbsC* vector

[a] From Ref 53.
[b] From Ref. 54.

TABLE 2
Oligonucleotides and plasmids

Oligos and plasmid	Description[a,b]	Source
Plasmids		
pGEX-SN	*E. coli* expression vector, Amp^r	Ref. 10
pGEX-SN_*fbsC*	pGEX-SN expression vec to r carrying *FbsC* (residues 37 to 386)	Ref. 36
pTCV_TetO	GBS (anhydro)tetracycline inducible expression vector, $P_{xyl/tetO}$ promoter, Erm^R, $K_m{}^R$	This study
pG1ts	Thermosensible shuttle vector, Erm^R	Ref. 55
pG1_Δ*fbcC*	pG1ts with *fbsC* in frame deletion cassette	This study
pTCV_TetO_*fbcC*	pTCV_TetO with the full lenght *fbsC* ORF	This study
Oligos		
gbs0791_BamH1	TTTGGATCCTAATGGCAGCAAGTGCACAACAA	
gbs0791_NotI	TTTTTTTGCGGCCGCCACTACCAACAAGGGCAGTTTTA	
383_EcoRI	AGATGAATTCCCAGACTTTTACCCTTACCAG	
384_Δ0791	GTGTCTAAAGACCCAAGCTTCTAACCGGTTAAGTTTTTATTACG	
385_Δ0791	CGTAATAAAAACTTAACCGGTTAGAAGCTTGGGTCTTTAGACAC	
386_BamHI	AGTAGGATCCAAACCGGAATATTACGATGCTTA	
562	TCCCCTTTACCATTGTCGAATAG	
563	ATTATTGGCAAACAGCTGATCAC	
389	TCAATTGATGGAAAATCAAAGG	
390	TTTAATTGGTGCTGTTGGTTTC	
pRPF185_Eco	TTATGAATTCTTAAGACCCACTTTCAC	
pRPF185_Bam	CTGCAGGATCCCAGATCTGTTAACGC	
537_BamHI	TGATGGATCCTTCTGGAGGAAAATAGTAATGAATAAATC	
538_PstI	TGATCTGCAGTGTGTCTAAAGACCCAAGCTTC	

[a] Erm^R, erythromycin resistance; $K_m{}^R$, kanamycin resistance; Amp^r, ampicillin resistance.
[b] Underlined nucleotides represent restriction sites.

required instead (15). Moreover, FbsA expression promotes growth in human blood (14) and mediates platelet aggregation, suggesting a role of this protein in GBS-induced endocarditis (17). Recently, it was reported that LP*X*TG glycoproteins Srr1 and Srr2 also contribute to fibrinogen binding (18) and that Srr1 mediated invasion of brain vascular endothelial cells and translocation through the blood-brain barrier (19).

Very recently, a novel fibronectin-binding protein, named BsaB, was identified in GBS strains (20). However, its interaction with fibrinogen was not studied (20) and we describe here that BsaB is in fact a specific fibrinogen-binding protein that we renamed FbsC, which is encoded by the *gbs0791* locus in strain NEM316. FbsC, which bears two immunoglobulin-like tandem repeat domains and a C-terminal cell wall-anchoring motif, was found here to mediate fibrinogen binding, biofilm formation, and invasion of epithelial and brain endothelial cells by GBS. Collectively, our data indicate that FbsC is an important virulence factor and a potential target for strategies aimed at controlling GBS infections.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Reagents—The following reference GBS strains (21) were used: NEM316 (serotype III, CC23), 6313 (serotype III, CC 23), BM110 (serotype III, CC17), COH1 (serotype III, CC17), A909 (serotype Ia, CC1), and 2603V/R (serotype V, CC19). The relevant characteristics of the other bacterial strains and plasmids used in this study are summarized in Table 1. GBS were grown at 37 °C in Todd-Hewitt broth (Difco Laboratories) or in Carey's chemically defined medium (22). Antibiotics were used at the following concentrations for *Escherichia coli:* ticarcillin, 100 μg/ml; erythromycin, 150 μg/ml; kanamycin, 25 μg/ml; and for GBS: erythromycin, 10 μg/ml; kanamycin, 500 μg/ml. Anhydrotetracycline (Sigma or Clontech) for gene induction in GBS was used at 500 ng/ml. Human fibrinogen was prepared as previously described (17). Human fibronectin and plasminogen were purchased from Calbiochem and bovine serum albumin was purchased from Sigma.

DNA Manipulation and Mutant Construction—Purification of GBS genomic DNA and *E. coli* plasmid DNA was performed on Qiagen columns following the manufacturer's instructions (DNeasy Blood and Tissue kit and Qiaprep Spin Minipreps kit, respectively). The oligonucleotides used in this study were provided by Eurofins MWG Operon or Sigma and are listed in Table 2. Analytical PCR was used standard *Taq* polymerase (Invitrogen). Preparative PCR for cloning and PCR for sequencing were carried out with a high fidelity polymerase (MyFi or Phusion DNA polymerase, Bioline and Thermo Scientific, respectively). Sanger sequencing was carried out at GATC Biotech.

The pG1_Δ*FbsC* deletion vector was constructed as described (23), using a splicing by overlap-extension method (24) with primers 383_EcoRI + 384_Δ*0791* and 385_Δ*0791* + 386_BamHII. After GBS transformation with pG1_Δ*fbsC* and selection of pG1_Δ*fbsC* integration and de-recombination events, marker-less deletion of *fbsC* was confirmed on genomic DNA with primers 562 + 563 (positive PCR product in case of *fbsC* deletion) and 389 + 390 (positive PCR product in case of a WT *fbsC* gene). The *fbsC* deletion was further confirmed by Sanger sequencing of the 562 + 563 PCR product. The multicopy shuttle vector pTCV_TetO was constructed to allow anydrotetracycline-inducible expression in GBS. This vector is based on the TetR-controlled $P_{xyl/tetO}$ promoter developed in *Staphylococcus aureus* (25) and *Clostridium difficile* (26). We amplified the TetR activator and the $P_{xyl/tetO}$ promoter from the pRPF185 vector (26) with primers pRPF185_Eco and pRPF185_Bam. The purified PCR product was digested by EcoRI and BamHI and cloned into the GBS shuttle vector pTCV-erm (27) to give pTCV_TetO. A PCR product containing the full-length *fbsC* ORF (1539 bp), the 18-bp sequence downstream of the *fbsC* start codon (to include the *fbsC* native ribosome binding site), and 31 bp upstream of the *fbsC* stop codon was obtained with primers 537_BamHI and 538_PstI. The purified PCR product was digested by BamHI and PstI and cloned into pTCV_TetO to give pTCV_TetO_*fbsC*. The full-length insert was sequenced to confirm the absence of mutations. The pTCV_TetO_*fbsC* was introduced in GBS by electroporation and transformants were selected on TH agar supplemented with kanamycin.

Production of Recombinant FbsC—Recombinant FbsC was produced as described (28, 29). Briefly, the *fbsC* gene was amplified using primers gbs0791_BamHI and gbs0791_NotI (Table 2) and cloned into the pGEX-SN bacterial expression vector (30). The corresponding pGEX-SN_FbsC allows the expression of the recombinant FbsC fused to a glutathione *S*-transferase (GST) tag at its amino-terminal end. After induction, the recombinant protein, designated as GST-FbsC, was purified from the cytoplasm of bacterial cells using affinity chromatography (29). Recombinant GST was produced and purified using the same method and used as a negative control.

Production of Anti-FbsC Antisera—CD1 mice (5 weeks old, Charles River Labs) were injected intraperitoneally with 20 μg of GST-FbsC or GST in complete (first injection) or incomplete (second and third injections) Freund's adjuvant emulsions (in a total volume of 0.2 ml) on days 0, 14, and 28. The use of complete Freund's adjuvant in the first immunization was justified by our previous observations that high-titer sera were more consistently obtained with this adjuvant, as compared with other less "inflammatory" adjuvants such as alum. However, care was taken to minimize discomfort to the animals by injecting a low volume (0.1 ml, containing 0.05 mg of mycobacteria) of the oily component of the emulsion and by using sterile solutions and techniques to prepare it. Under these conditions, no significant abdominal distension or other complications at the injection site were observed throughout the experimental period. The mice were bled at 2 weeks after the last immunization, and the sera were tested for reactivity to the purified antigen using ELISA and Western blot assays.

Bacterial Extracts and Immunoblots—To analyze secreted proteins, supernatants from 40 ml of Carey's chemically defined medium cultures were collected at mid-exponential phase ($A_{600\ nm}$ = 0.5), filter sterilized, and concentrated 50-fold using centrifugation in Amicon Ultra-15 tubes (Millipore). Cell wall extracts were obtained as described (28), after digestion of purified cell walls with mutanolysin (Sigma) in osmo-protective buffer. Hot SDS extraction of whole bacterial cells was performed as previously described (31). After SDS-PAGE, proteins were transferred to nitrocellulose membranes and FbsC was detected using mouse anti-GST-FbsC serum followed by alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma), as described (31). The amounts of proteins loaded on gels were calculated from protein concentrations, as determined by the Bradford assay using BSA as a standard. Loading controls consisted of parallel Coomassie-stained gels. For Far Western blots, fibrinogen (10 μg) was run on 12% acrylamide gels, transferred on nitrocellulose, and overlaid with 0.5 μM GST-FbsC or GST in 1% of nonfat dry milk supplemented with 2% Tween 20. Complex formation was detected using goat anti-GST IgG (1:4,000) followed by horseradish peroxidase-conjugated anti-goat IgG (1:5,000). Five μg of GST-FbsC or GST were also run on 12% acrylamide gels, transferred on nitrocellulose, and overlaid with fibrinogen (1 μg/ml). Complex formation was detected using anti-fibrinogen mouse monoclonal antibody 1F3 and alkaline phosphatase-conjugated goat anti-mouse IgG, as described (31).

Analysis of FbsC Binding to Extracellular Matrix Components by ELISA—Extracellular matrix components, including fibrinogen, fibronectin, plasminogen, and BSA, used as a control, were coated onto microtiter wells overnight at 4 °C in 0.1 M carbonate buffer (pH 9.0). The wells were washed with phosphate-buffered saline (PBS) supplemented with 0.05% Tween 20, blocked with PBS supplemented with 0.01% Tween 20 and 1% nonfat dry milk for 2 h at 20 °C, and incubated with 5 μg/ml of GST-FbsC or GST for 1 h. Complex formation was detected with goat anti-GST (1:4,000; GE Healthcare), followed by the addition of alkaline phosphatase-conjugated rabbit anti-goat IgG (1:5,000; Sigma). For the competitive ELISA, GST-FbsC was co-incubated with the indicated amounts of soluble inhibitors for 15 min at 20 °C before the addition to fibrinogen-coated plates.

Immunofluorescence Microscopy and Flow Cytometry Analysis—Binding of fibrinogen or anti-FbsC antibodies to the bacterial cell surface was visualized using immunofluorescence microscopy on an Axio Observer microscope equipped with a structured illumination apparatus (Apotome), using previously described methods (29). Briefly, GBS strains grown to the stationary phase in Todd-Hewitt broth were washed in PBS, dried on glass coverslips, fixed with 3.7% formaldehyde, and then blocked using PBS supplemented with 5% dry milk. For fibrinogen-binding studies, slides were sequentially incubated with fibrinogen (50 μg/ml) in PBS supplemented with 1% milk (mPBS) and with an anti-fibrinogen mouse monoclonal antibody (1F3, Abcam, diluted 1:5,000 in 1% mPBS). The slides were then treated with FITC-conjugated goat anti-mouse IgG (Sigma) diluted 1:1,000 in 1% mPBS in the presence of DAPI

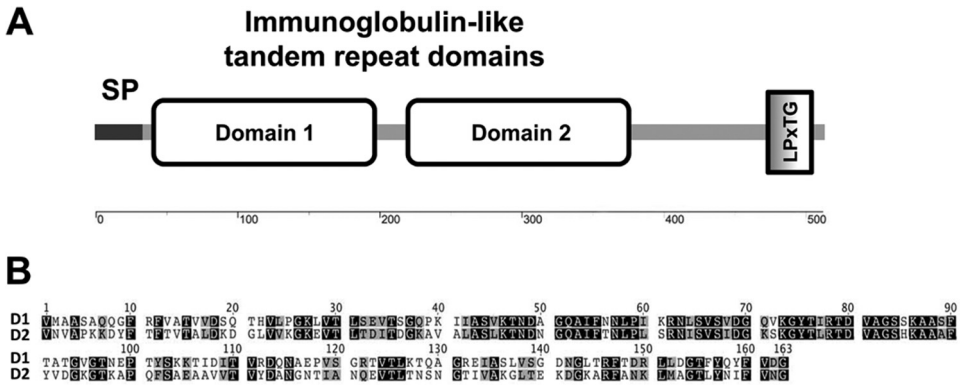

FIGURE 1. **Schematic representation of FbsC.** *A*, the relevant characteristics of FbsC are a signal peptide (*SP*), two tandemly repeated domains (*D1* and *D2*), and a carboxylic cell wall anchoring (LP*X*TG) motif; *B*, sequence comparison of domains 1 and 2, displaying 45.5% identity.

(0.5 μg/ml, Sigma). To visualize surface-expressed FbsC, slides were incubated with anti-GST-FbsC or anti-GST serum diluted 1:100 followed by FITC-conjugated goat anti-mouse IgG (diluted 1:1,000, Sigma). Flow cytometry immunofluorescence analysis was also used to visualize FbsC expression on the bacterial surface, using previously described methods (28–29). Briefly, bacteria grown to the early-log phase were sequentially incubated with mouse anti-GST-FbsC antiserum (diluted 1:100) and FITC-conjugated goat anti-mouse IgG. Fluorescent bacteria were analyzed with FACSCantoII flow cytometer using the FlowJo software (both from BD Biosciences).

Surface Plasmon Resonance—Surface plasmon resonance studies were performed using the BIAcore X system (GE Healthcare). To measure K_D values of fibrinogen binding to recombinant GST-FbsC, goat anti-GST antibody (30 μg/ml) dissolved in 10 mM sodium acetate buffer (pH 5.0) was immobilized onto a carboxy-derivatized sensor chip. GST-FbsC (500 nM) was passed over a flow cell, whereas GST alone was passed in a reference cell. Human fibrinogen was then flowed over the surface of both flow cells at concentrations ranging from 2.92 to 750 nM at a rate of 20 μl/min. Assay channel data were subtracted from reference flow cell data to eliminate the effects of nonspecific interactions. The data were analyzed using the BIA evaluation software version 3.0. A plot of the level of binding (response units) at equilibrium against analyte concentration was used to determine K_D values.

Adhesion and Invasion—Human epithelial (Caco-2 and A549) cell lines were obtained from the American Type Culture Collection. The human brain endothelial cell line hCMEC/D3 (32) was provided by P.O. Couraud (INSERM, Paris, France). Cell lines were cultured as previously described (29, 32). The adherence and invasion assays were performed as described (29). Briefly, bacteria were grown to the mid-log phase and added to confluent monolayers at a multiplicity of infection of 25. After a 2-h incubation, monolayers were extensively washed with PBS to remove the non-adherent bacteria, lysed, and plated to enumerate cell-associated bacteria. For the invasion assay, after washing, the monolayers were further incubated for 1 h with medium supplemented with penicillin and streptomycin (200 units/ml and 200 μg/ml, respectively) to kill extracellular bacteria. Bacterial adherence and invasion were calculated as follows: recovered cfu/initial inoculum cfu $\times$ 100. Where indicated, bacteria were pre-treated with exogenous fibrinogen (50 μg/ml) for 30 min at 20 °C before addition to the cell monolayers.

Bacterial Attachment to Immobilized Fibrinogen—Microtiter plates were coated overnight at 4 °C with fibrinogen at the indicated concentrations in PBS. The wells were washed three times with PBS before the addition of 10^5 cfu of GBS to each well, and the plates were then incubated for 1 h at 37 °C. After extensive washing, the wells were treated with trypsin (2.5 mg/ml, Sigma) for 10 min at 37 °C to release the attached bacteria, which were then enumerated by agar plate counts. For inhibition experiments, fibrinogen-coated plates or bacteria were pretreated for 15 min at 20 °C with the indicated inhibitors before the assay.

Biofilm Formation Assays—GBS strains grown overnight in Todd-Hewitt broth were diluted in Luria broth supplemented with 1% glucose (Difco Laboratories) to reach a final $A_{600\ nm}$ of 0.1. Next, 100 μl were added to each well of 96-well polystyrene flat-bottom microtiter plates, whereas wells filled with non-inoculated growth medium were included as negative controls. Plates were incubated without shaking at 37 °C for 24 h in 5% CO_2. Before biofilm quantification, bacterial growth was assessed by measuring $A_{600\ nm}$ values and medium, including any unattached bacteria, was decanted from the wells. These were then rinsed with PBS and air dried, and adherent bacteria were stained for 15 min with a 0.1% (w/v) solution of crystal violet (Sigma). After rinsing with PBS, bound dye was released from stained cells using ethanol/acetone (80:20) and quantified by measuring $A_{590\ nm}$ values. For SE observations, GBS strains were diluted and incubated as above, and seeded on coverslips in 24-well plates. After incubation, the slides were fixed in gluteraldehyde/formaldeyde (both at a 2.5% concentration), dehydrated, and imaged by SE according to standard procedures (33).

Immunoprotective Activities of FbsC Immunization and Virulence Studies—To study the protective activity of FbsC immunization, CD1 mice (5 weeks old, Charles River Labs) were injected intraperitoneally with 20 μg of GST-FbsC or GST in complete (first injection) or incomplete (second and third

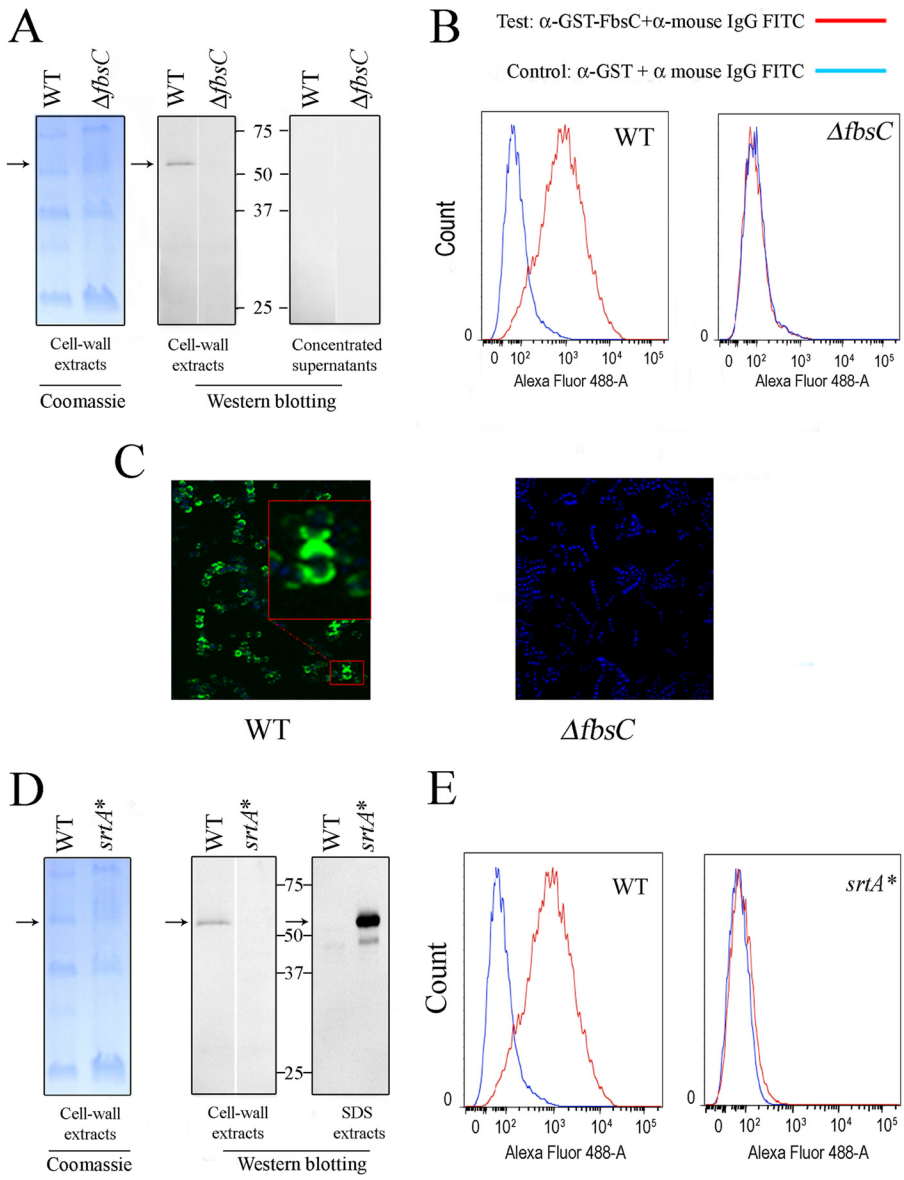

FIGURE 2. **Western blot and immunofluorescence analysis of parental wild-type NEM316 (*WT*) and its *fbsC* deletion (Δ*fbsC*) or sortase A-defective (*srtA**) mutants using a mouse antiserum raised against the GST-FbsC fusion protein (α-GST-FbsC).** *A*, Western blot analysis: 10 μg (protein weight) of cell wall extracts or concentrated supernatants from WT or Δ*fbsC* strains were run on PAGE gels, stained with Coomassie or transferred to nitrocellulose, and probed with mouse anti-GST-FbsC serum followed by alkaline phosphatase-conjugated anti-mouse IgG. The *numbers between the panels* indicate the $M_r \times 1000$ of M_r standards. The *arrows* indicate the positions corresponding to the FbsC protein bands. *B*, immunofluorescence flow cytometry analysis of WT or Δ*fbsC* strains incubated with mouse anti-GST-FbsC (*red line*), or control anti-GST serum (*blue line*), followed by FITC-conjugated anti-mouse IgG (α-mouse IgG FITC). *C*, immunofluorescence microscopy analysis of WT or Δ*fbsC* strains incubated with mouse anti-GST-FbsC, followed by FITC-conjugated anti-mouse IgG. *D* and *E*, analysis of FbsC expression in the *srtA** mutant strain using anti-GST-FbsC mouse sera. *D*, Western blot analysis; 10 μg (protein weight) of cell wall digests or whole cell SDS extracts from WT or *srtA** strains were run on PAGE gels, stained with Coomassie, or transferred to nitrocellulose and probed with mouse anti-GST-FbsC serum followed by alkaline phosphatase-conjugated anti-mouse IgG. The *numbers between the panels* indicate the molecular mass of protein standards in kDa. The *arrows* indicate the positions corresponding to the FbsC protein bands. *E*, immunofluorescence flow cytometry analysis of WT or *srtA** strains incubated with mouse anti-GST-FbsC serum (*red line*) or control anti-GST serum (*blue line*) followed by FITC-conjugated anti-mouse IgG.

injections) Freund's adjuvant emulsions (in a total volume of 0.2 ml) on days 0, 14, and 28. Three weeks after the last immunization, mice were challenged intraperitoneally with GBS strain NEM316 (2×10^8 cfu). Mice were monitored at least once a day for lethality and signs of disease for a total of 14 days after challenge. Animals with signs of irreversible sepsis were humanely euthanized and their organs were cultured to confirm GBS as the cause of disease. In further experiments, GBS-infected mice were sacrificed at 24 or 48 h after infection to collect blood, brains, and kidneys. The number of cfu was measured in organ homogenates using standard methods (30). To measure the virulence of GBS mutants, 8-week-old CD1 mice were infected intraperitoneally or intravenously with the indicated bacterial doses. Survival and organ cfu were determined as described above.

RESULTS

FbsC Is Anchored to the Cell Wall by Sortase A—Gbs0791 (thereafter referred to as FbsC, standing for fibrinogen binding surface protein C) is one of the five genes encoding LP*X*TG

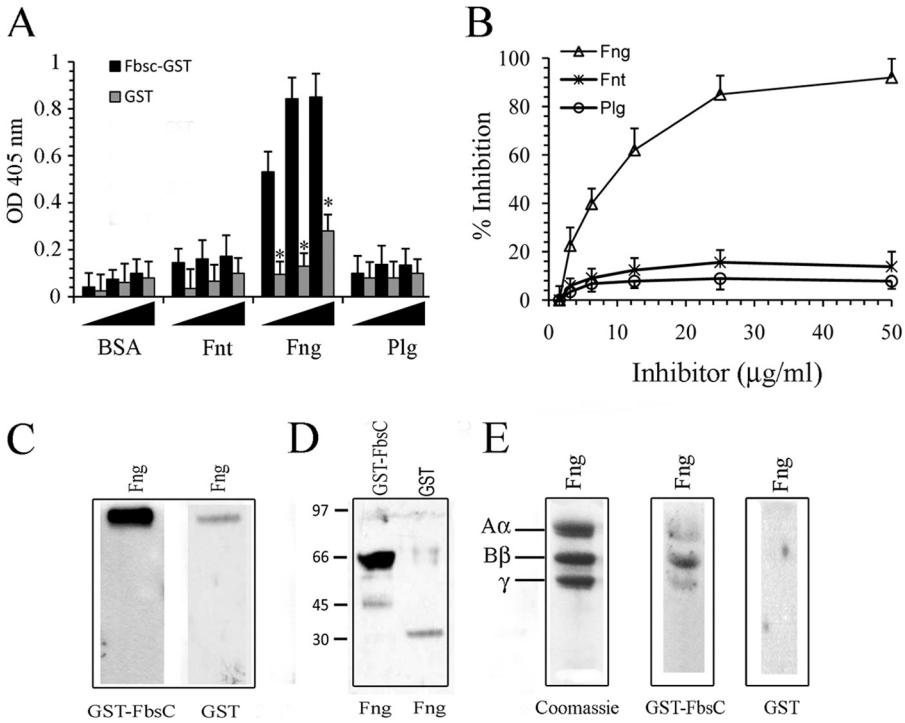

FIGURE 3. **Binding of recombinant FbsC to human fibrinogen.** *A*, ELISA in which plates were sensitized with increasing doses (1, 5, and 10 μg/ml) of bovine serum albumin (BSA), human fibronectin (*Fnt*), fibrinogen (*Fng*), or plasminogen (*Plg*) followed by incubation with recombinant GST-FbsC or GST (both at a concentration of 10 μg/ml). Bound recombinant proteins were detected using alkaline phosphatase-conjugated anti-GST IgG. *Columns* and *bars* indicate mean ± S.D. from three independent experiments. *, $p < 0.05$ by analysis of variance and Student's Neuman Keuls test. *B,* competitive ELISA using plates sensitized with fibrinogen: a fixed GST-FbsC concentration (10 μg/ml) was mixed with soluble fibrinogen, or with fibronectin or plasminogen as inhibitor, at the concentrations indicated in the horizontal axis, before the addition to plates sensitized with immobilized fibrinogen (5 μg/ml). Bound GST-FbsC was detected using alkaline phosphatase-conjugated anti-GST IgG. *Points* and *bars* indicate mean ± S.D. from three independent experiments. *C*, Far Western blot analysis in which fibrinogen (10 μg) was run on PAGE gels under non-reducing conditions, transferred to nitrocellulose membranes, and probed using GST-FbsC or GST. Bound recombinant proteins were detected using alkaline phosphatase-conjugated anti-GST IgG. *D*, Far Western blot analysis in which 5 μg of GST-FbsC or GST were run on PAGE gels, transferred to nitrocellulose membranes, and probed using fibrinogen. Bound fibrinogen was detected using mouse anti-fibrinogen IgG followed by alkaline phosphatase-conjugated anti-mouse IgG. *Numbers* indicate the molecular mass of protein standards in kDa. *E*, Coomassie staining and Far Western blot analysis in which fibrinogen (10 μg) was run on PAGE gels under reducing conditions, transferred to nitrocellulose membranes, and probed using GST-FbsC or GST followed by alkaline phosphatase-conjugated anti-GST IgG.

surface proteins whose transcription is strongly repressed by the pleiotropic two-component regulatory system CovRS (also called CsrRS) in GBS strain NEM316 (34), a finding recently validated at the protein level (35, 36). FbsC possesses two bacterial immunoglobulin-like tandem repeat domains and a C-terminal cell wall-anchoring motif (LP*X*TG, Fig. 1). To characterize its biological functions, we constructed an isogenic mutant strain bearing an in-frame deletion in the *fbsC* gene (Δ*fbsC*, Table 1). Viability, morphology, and growth in Todd-Hewitt broth of the mutant were similar to those of the wild-type strain (not shown). Western blot analysis performed with specific polyclonal mouse serum (pAb) directed against a recombinant form of FbsC in fusion with GST (GST-FbsC) revealed that the antigen was detected in the cell wall extracts but not in the concentrated supernatants of the NEM316 GBS strain (Fig. 2*A*), whereas no bands were observed using control anti-GST serum (not shown). The specificity of anti-GST-FbsC pAb was confirmed by the absence of immunoreactive bands in the cell wall extracts of a Δ*fbsC* strain (Fig. 2*A*). Flow cytometry and fluorescent microscope analysis of NEM316 WT and Δ*fbsC* mutant stained with anti-GST-FbsC pAb consistently revealed that this protein was present only at the surface of the WT strain (Fig. 2, *B* and *C*). The antigen was mainly localized to the cell poles (Fig. 2*C*), in a pattern reminiscent of that observed with other cell wall proteins of GBS (37).

To demonstrate that FbsC is anchored to the cell wall by the housekeeping sortase A (SrtA), cell wall digests and SDS extracts from NEM316 WT and *srtA** mutant strains were analyzed by Western blot analysis with the specific mouse pAb. FbsC was detected in the cell wall digests of the WT strain but not in those of the *srtA** mutant (Fig. 2*A*). In contrast, we observed that this protein was only present in SDS extracts from the *srtA** mutant (Fig. 2*D*), which is unable to covalently anchor LP*X*TG surface adhesins to the peptidoglycan and retains the unprocessed adhesins into the bacterial membranes (31). Presence of FbsC in cell wall digests of the WT strain, but not in the SDS extracts, indicated that it is covalently linked to the cell wall. Flow cytometry analysis confirmed the display of FbsC at the surface of the WT strain only (Fig. 2*E*). Collectively, these results demonstrate that FbsC is a surface-exposed protein covalently linked to the cell wall by a sortase A-dependent mechanism.

Last, FbsC expression was tested by flow cytometry analysis in additional GBS reference strains representative of the main clonal complex (CC) lineages. FbsC was found on the surface of A909 (serotype Ia, CC1), 2603V/R (serotype V, CC19), and

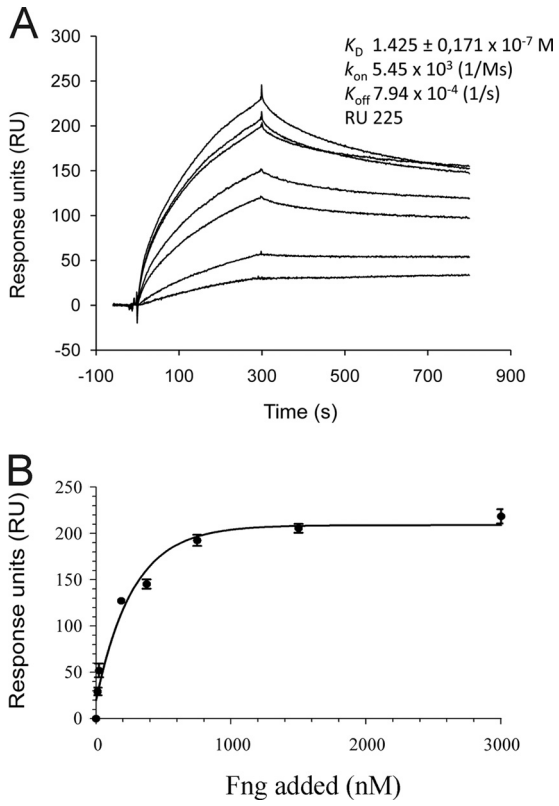

FIGURE 4. **Analysis of fibrinogen binding to recombinant GST-FbsC using surface plasmon resonance.** GST-FbsC or GST were immobilized on anti-GST-coated sensor chips. Increasing concentrations of fibrinogen (*Fng*) were then flowed through the cells. Assay channel data were subtracted from reference flow cell data to eliminate the effects of nonspecific interactions. The data shown are from one experiment, representative of three and performed in triplicate.

6313 (serotype III, CC23) strains, but not on strains BM110 and COH1 that are representative of the CC17 lineage (not shown). Accordingly, analysis of genomic sequences of 20 CC17 isolates, including BM110 and COH1, revealed deletion of an adenine at position 600 of the *fbsC* gene in all strains (data not shown).

FbsC Binds Fibrinogen in Vitro—As mentioned above, FbsC contains two repeated motifs forming a bacterial Ig-like domain, a feature found in many bacterial surface-exposed proteins that bind to host components, in particular those present in the extracellular matrix. We thus tested the ability of FbsC to bind to plasminogen, fibrinogen, and fibronectin, which are ubiquitous components of human tissues. In an ELISA in which these extracellular matrix components were immobilized on plastic plates, recombinant GST-FbsC was able to specifically bind fibrinogen, but not to plasminogen, fibronectin, or bovine serum albumin (Fig. 3*A*). Moreover, when GST-FbsC was preincubated with different concentrations of soluble fibrinogen, but not plasminogen or fibronectin, a dose-dependent inhibition of binding to immobilized fibrinogen was observed (Fig. 3*B*). The binding of FbsC to fibrinogen was further confirmed in Far Western blotting experiments where these proteins were used alternatively as bait or pray (Fig. 3, *C* and *D*). FbsC binding was predominantly localized to the fibrinogen Bβ chain and only weakly to the Aα and γ chains (Fig. 3*E*). The affinity of interaction between fibrinogen and FbsC was investigated using BIAcore (Fig. 4). To this end, FbsC-GST was immobilized on a sensor chip, over which different concentrations of fibrinogen were subsequently flowed. The obtained sensorgrams yielded a $K_D = 1.425 \pm 0.171 \times 10^{-7}$ M for fibrinogen-FbsC interaction.

FbsC Expression Is Required for Bacterial Binding to Fibrinogen—Fibrinogen is present in the host both as a surface-bound molecule (*e.g.* on the surface of epithelial cells or in the extracellular matrix) and in soluble form (*e.g.* in the blood). To test the contribution of FbsC on the binding of GBS to fibrinogen, we first compared the ability of NEM316 WT and Δ*fbsC* mutant strains to adhere to plastic plates coated with fibrinogen, fibronectin, or plasminogen. Under these conditions, adherence of the Δ*fbsC* mutant to fibrinogen, but not fibronectin or plasminogen, was almost completely abrogated in comparison to the WT strain (Fig. 5*A*). Residual adherence of the Δ*fbsC* mutant to fibrinogen was similar to that of the *srtA** mutant, which is unable to covalently anchor LP*X*TG surface adhesins to the peptidoglycan. Genetic complementation of the Δ*fbsC* mutant restored FbsC expression (Fig. 5*B*) and the WT ability to bind immobilized fibrinogen (Fig. 5*C*), thus confirming that the inability of the mutant strain to bind fibrinogen is due to deletion of the *fbsC* gene. Moreover, binding of WT bacteria to immobilized fibrinogen was almost completely abrogated in the presence of soluble fibrinogen, GST-FbsC, or anti-GST-FbsC pAb (Fig. 5*D*). Collectively, these results demonstrate that, in the tested conditions, FbsC is a major fibrinogen-binding protein coded by NEM316.

FbsC Expression Increases Biofilm Formation—Bacterial growth on epithelial surfaces or on implantable devices can be associated with the elaboration of a self-formed matrix or a biofilm. We investigated whether the ability of GBS to form biofilms depends on the expression of FbsC. To this end, GBS strains were grown in polystyrene plates and bacterial biomass formation was measured by crystal violet staining. As expected, the *srtA** mutant produced minimal biofilm, whereas robust biomass formation was observed using wild type NEM316 (Fig. 6*A*). Under these conditions, biofilm production by the Δ*fbsC* deletion mutant was intermediate between that of NEM316 WT and the *srtA** mutant (Fig. 6*A*). Scanning electron microscopy of the Δ*fbsC* biofilm reveals the formation of mainly isolated bacterial chains in contrast to WT biofilm forming tightly compact clusters of bacterial chains surrounded by a dense extracellular matrix (Fig. 6*B*). This data indicated that FbsC plays a significant role in biofilm formation, probably by promoting bacterial aggregation on solid surfaces.

FbsC Expression Is Necessary for Adhesion to and Invasion of Epithelial Cells—Attachment to and invasion of epithelial cells by GBS play a crucial role in the initial stages of the infection process. Alveolar and intestinal epithelia are considered as likely entry sites in early- and late-onset GBS disease (38, 39). To test whether FbsC contributes to interactions between GBS and epithelial cells, we compared the binding of NEM316 WT, Δ*fbsC,* and *srtA** mutant strains to Caco-2 (intestinal) and A549 (pulmonary) human epithelial cell lines. The adherence of the Δ*fbsC* strain to CaCo-2 or A549 cells was significantly reduced compared with NEM316, indicating that FbsC is required for optimal bacterial adherence (Fig. 7*A*). The Δ*fbsC* mutant, however, was more adherent to both cell types than the *srtA**

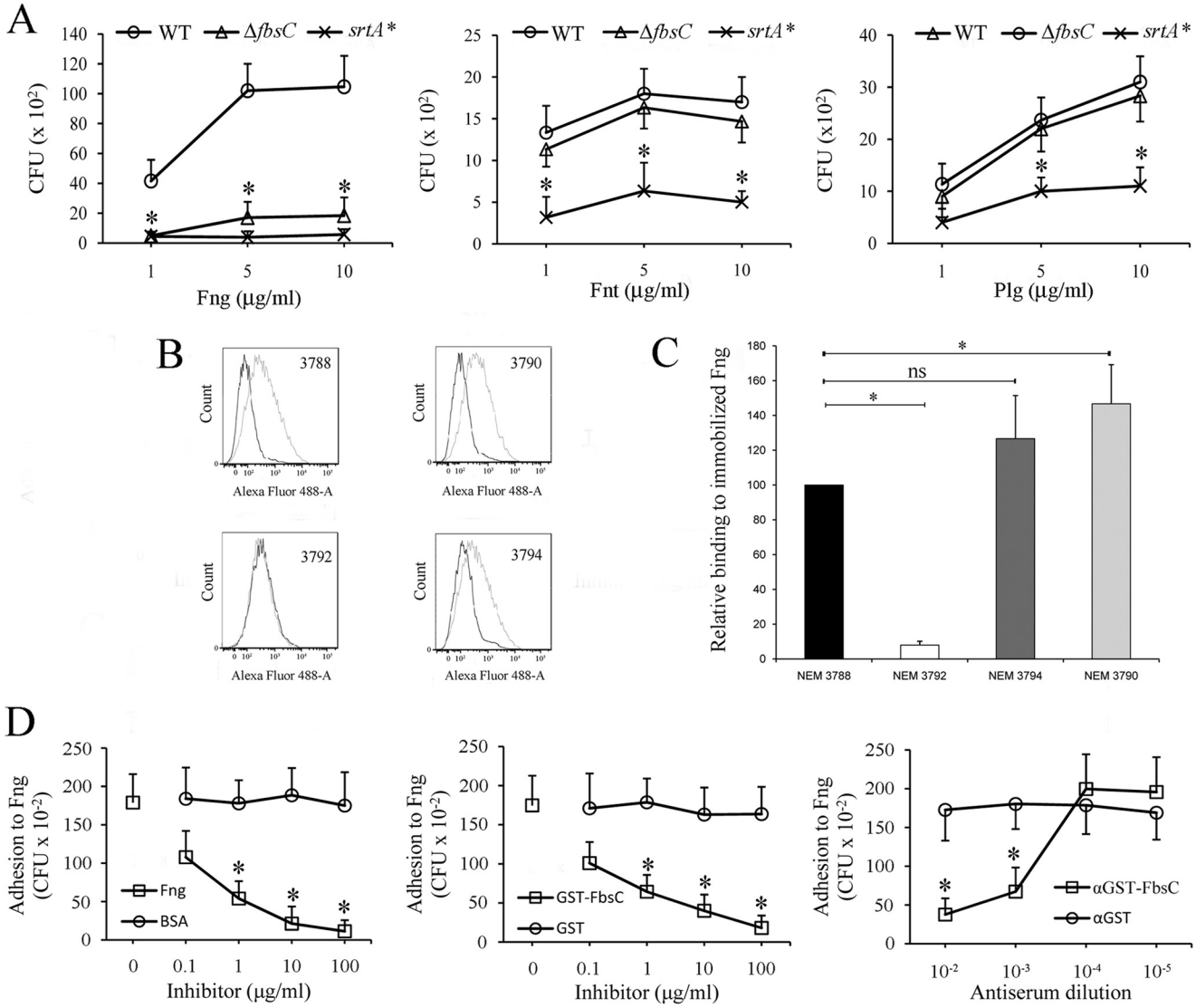

FIGURE 5. **Contribution of FbsC to the overall ability of GBS to bind fibrinogen.** Wild-type NEM316 (*WT*) was compared with its *fbsC* deletion (Δ*fbsC*) or sortase A-defective (*srtA**) mutants. * = $p < 0.05$ by analysis of variance and Student's Neuman Keuls test. *A*, binding of GBS strains to fibrinogen (*Fng*), fibronectin (*Fnt*), or plasminogen (*Plg*) immobilized on plastic plates at the concentrations indicated in the *horizontal axis*. Shown are mean ± S.D. of cfu from three independent experiments. *B*, effects of complementation of the *fbsc* deletion on GBS binding to soluble fibrinogen (1 μg/ml). Bound fibrinogen was visualized with mouse anti-fibrinogen IgG followed by FITC-conjugated anti-mouse IgG. *NEM3788*, WT strain containing the pTCV_TetO (empty) vector; *NEM3790*, WT strain containing the pTCV_TetO_ *fbsC* vector and overexpressing the protein; *NEM3792*, Δ*fbsC* containing the pTCV_TetO (empty) vector; *NEM3794*, Δ*fbsC* complemented with the pTCV_TetO_ *fbsC* vector. *C*, effects of complementation of the *fbsC* deletion on adherence to immobilized fibrinogen (5 μg/ml). The results shown are mean ± S.D. of cfu counts from three independent experiments. *D*, inhibition of GBS binding to immobilized fibrinogen (5 μg/ml) by soluble fibrinogen, recombinant FbsC, or anti-FbsC antibodies used at the indicated concentrations. The results shown are mean ± S.D. of cfu counts from three independent experiments.

mutant, suggesting that besides FbsC other LP*X*TG-anchored adhesins play a role in adherence to epithelial cells. Complementation of the *fbsC* mutation with the wild-type allele restored bacterial adherence to A549 cells (Fig. 7*A*). As shown for adherence, invasion of both epithelial cell lines by the Δ*fbsC* mutant was significantly reduced, but was still higher than that observed with the *srtA** mutant (Fig. 7*B*). Moreover, adhesion to and invasion of epithelial cells by WT bacteria was almost completely abrogated in the presence of GST-FbsC (Fig. 7*C*). Collectively these results indicate that GBS interactions with epithelial cells are largely dependent on surface-expressed FbsC. Because fibrinogen levels can increase at sites of injury and during inflammation of mucosal tissues (12), we also studied the interactions of GBS with A549 and Caco-2 cells after treatment of bacteria with fibrinogen. Fibrinogen pre-treatment of NEM316 WT, but not of the Δ*fbsC* mutant, was associated with a significant increase in both adherence and invasion (data not shown), suggesting that both processes largely depend on FbsC-mediated fibrinogen binding.

Role of FbsC in Invasion of Brain Endothelial Cells by GBS—Interaction with human brain vascular endothelial cells is considered a crucial step in the invasion of the blood-brain barrier by GBS (40–42). To investigate whether FbsC is involved in interaction with the endothelial cell line hCMEC/D3, we compared the adhesion properties of NEM316 WT with those of its isogenic mutant Δ*fbsC*. After 1 h, NEM316 WT efficiently adhered to these cells, whereas binding of the Δ*fbsC* mutant was significantly reduced (Fig. 7*D*). Preincubation of bacteria with

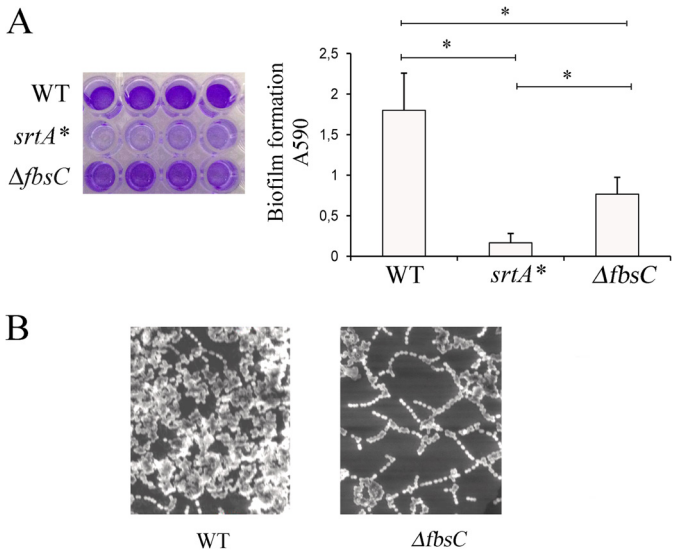

FIGURE 6. **Contribution of FbsC to the ability of GBS to form biofilm.** Wild-type NEM316 (WT) was compared with its *fbsC* deletion (Δ*fbsC*) or sortase A-defective (*srtA**) mutants. *A*, GBS strains were grown in liquid medium in 96-well polystyrene plates and biofilm was stained with crystal violet. *Left panel*, a crystal violet-stained microtiter plate; *right panel*, A_{595} measured after releasing crystal violet with ethanol/acetone from stained biofilms. *Column* and *bars* indicate the mean ± S.D. values from three independent experiments * = $p < 0.05$. *B*, scanning electron microscopy analysis of biofilm formation.

exogenous fibrinogen enhanced adherence of the WT strain, but not that of the Δ*fbsC* mutant. Similar effects were observed when studying invasion, which was significantly reduced using the Δ*fbsC* mutant compared with wild-type NEM316 (Fig. 7*D*). These data indicated that FbsC largely mediates *in vitro* adhesion to and invasion of brain vascular endothelial cells by GBS.

FbsC Is Required for GBS Pathogenicity—The virulence of the Δ*fbsC* mutant was compared with that of NEM316 WT using murine models of invasive infection. When adult mice were infected intraperitoneally with high (4×10^8 cfu) or low (2×10^8 cfu) doses of either strain, the Δ*fbsC* mutant displayed an attenuated virulence compared with the WT strain (Fig. 8, *A* and *B*). Notably, all mice infected with a high dose of the WT bacteria died within 2 days, whereas 90% of mice similarly infected with the Δ*fbsC* mutant survived at the end of the experiment (10 days) (Fig. 8*A*). With the lower inoculum, the lethality associated to the WT strain decreased to 63%, whereas no death was recorded with the mutant strain (Fig. 8*B*). By 24 h post-infection, cfu counts in the blood and kidney of mice infected with the low bacterial dose revealed more than one log difference between the WT and Δ*fbsC* mutant strains (Fig. 8*C*).

To test the hematogenous colonization of the brain by GBS, mice were infected intravenously with 4×10^8 cfu and blood and brain colony counts were obtained at 24 and 48 h post-infection. At both time points, decreased bacterial counts were measured in the blood and brains of mice injected with the Δ*fbsC* mutant compared with those injected the wild-type strain (Fig. 8*D*). These data indicated that FbsC plays an important role in the pathogenesis of invasive GBS disease by favoring systemic spreading of bacteria from the initial focus of infection to the invasion of organs.

FbsC-induced Immunoprotection—The surface exposition of FbsC and its contribution to GBS pathogenicity led us to test the protective potential of immunization with FbsC against GBS infection. Adult mice were immunized with recombinant GST-FbsC or GST (used as a negative control) and challenged intraperitoneally with 2×10^8 cfu of the NEM316 strain at 3 weeks after the last immunization. Under these conditions, immunization with GST-FbsC resulted in 80% (16 mice of 20) survival, whereas only 30% (6 mice of 20) of the GST-immunized animals survived ($p < 0.05$; Fig. 9*A*). In addition, blood, kidney, and brain colony counts were significantly lower in GST-FbsC-immunized mice at 24 h after challenge (Fig. 9*B*). These data indicated that immunization with FbsC significantly decreased GBS-induced lethality and *in vivo* bacterial pathogenicity.

DISCUSSION

Bacterial infections involve binding of pathogens to host ligands located on the surface of host cells and in the extracellular matrix (43–45). In several bacterial species, fibrinogen-binding proteins play crucial roles in pathogenesis by allowing bacteria to penetrate host barriers and spread in tissues (9, 46). GBS strongly interact with fibrinogen, a property that is more pronounced in strains isolated from invasive infections (10, 11, 13). To date, four structurally unrelated fibrinogen-binding proteins (FbsA, FbsB, Srr1, and Srr2) involved in virulence have been identified in GBS (14–16, 18, 19) and three of these are encoded by our prototype strain NEM316. Two additional GBS proteins (named Fib and SAG0242) were found to bind fibrinogen, but their properties have not been studied in depth (47). In this report, we describe a novel major fibrinogen-binding protein of GBS that was termed FbsC in analogy to FbsA and FbsB. On the amino acid level, FbsC does not exhibit significant similarity with other fibrinogen-binding proteins of GBS or other organisms, although its general architecture with two large internal tandemly repeated domains and a cell anchor motif is similar to that of other bacterial adhesins and virulence factors (48).

As expected for an LPXTG protein, FbsC is anchored to the cell wall by a sortase A-dependent mechanism and freely accessible on the cell surface. A recombinant FbsC protein specifically bound fibrinogen *in vitro* in a dose-dependent, saturable fashion with a dissociation constant value that was intermediate between those of FbsB (15) and FbsA (15, 49). FbsC is apparently essential for the overall ability of GBS to bind fibrinogen, because binding to both soluble and surface-associated fibrinogen was almost completely abrogated in a Δ*fbsC* deletion mutant. Plasmid-mediated expression of *fbsC* restored the capability of Δ*fbsC* to bind fibrinogen to wild-type levels, demonstrating that impaired fibrinogen binding of the mutant is caused by its *fbsC* deficiency. In the absence of FbsC, NEM316 adherence to and invasion of host cells, biofilm formation, and its virulence are significantly reduced. In particular, deletion of *fbsC* resulted in a significant decrease in the attachment to and invasion of lung and intestinal human epithelial cells. This may be relevant to the pathogenesis of neonatal infections, because lung and intestinal epithelia are the presumed bacterial entry sites in early- and late-onset GBS diseases, respectively (38, 39). Because epithelial cells express fibrinogen on their surface (50) it is likely that FbsC-dependent adherence and invasion are

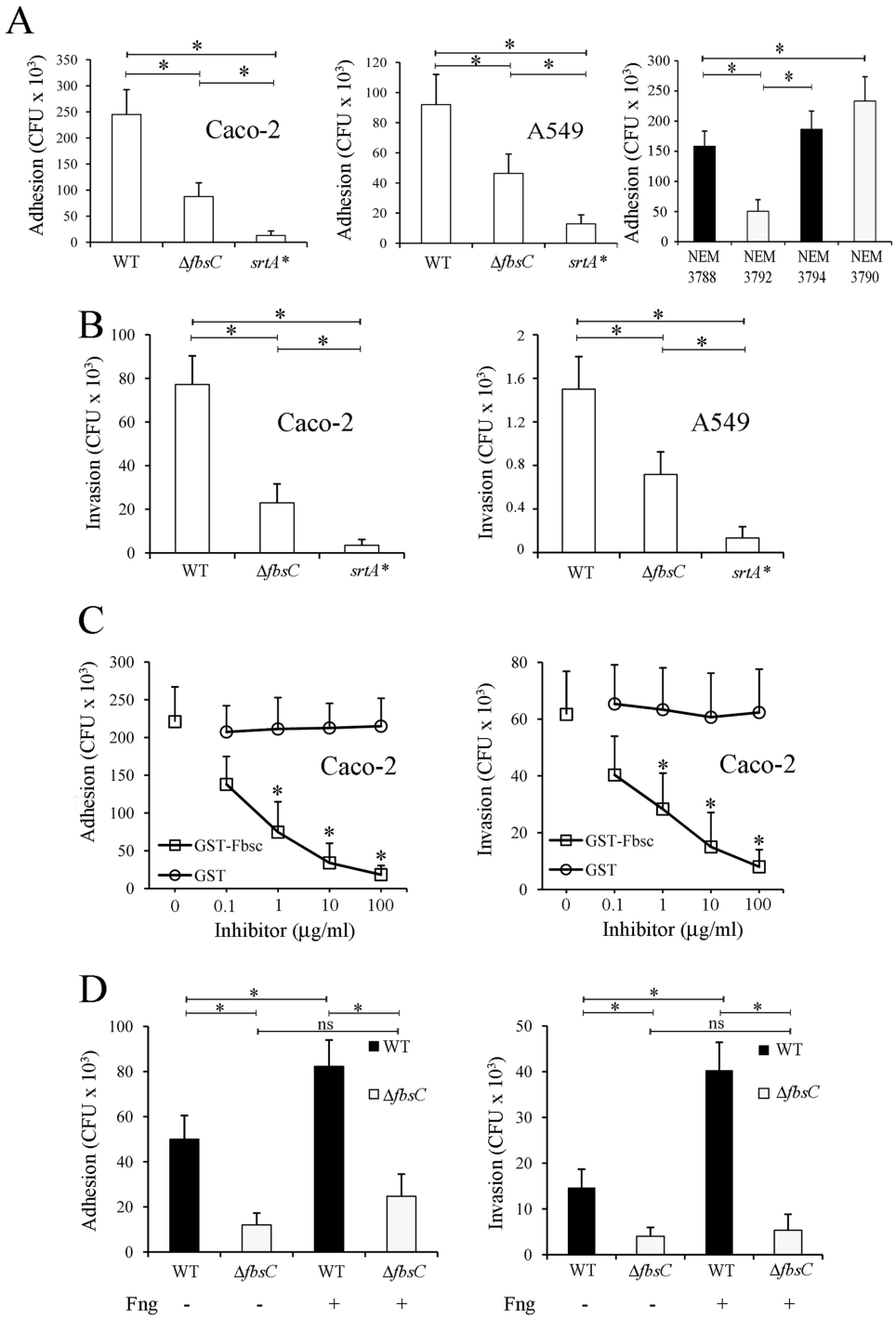

FIGURE 7. **Association to human epithelial and endothelial cell lines of wild-type NEM316 (*WT*) and its *fbsC* deletion (Δ*fbsC*) or sortase A (*srtA**)-defective mutants.** Each panel shows the mean ± S.D. of cfu counts from three independent experiments; *, $p < 0.05$ by analysis of variance and Student's Neuman Keuls test. *A*, adhesion to intestinal (*left panel*) and respiratory (*center panel*) epithelial cell lines; *right panel*, effects of complementation of the *fbsc* deletion on adherence to intestinal epithelial cells. *NEM3788*, WT containing the pTCV_TetO (empty) vector; *NEM3790*, WT containing the pTCV_TetO_ *fbsC* vector and overexpressing the protein; *NEM3792*, Δ*fbsC* containing the pTCV_TetO (empty) vector; and *NEM3794*, Δ*fbsC* complemented with the pTCV_TetO_ *fbsC* vector. *B*, invasion of intestinal (*left*) and respiratory (*right*) cell lines; *C*, inhibition of GBS adhesion to and invasion of intestinal epithelial cells by soluble recombinant GST-FbsC or GST, used at the indicated concentrations. *D*, interactions of GBS with the brain endothelial cell line hCMEC/D3 in the presence and absence of pretreatment of bacteria with fibrinogen.

mediated by GBS-fibrinogen interactions. This hypothesis is strengthened by our observations that pretreatment with fibrinogen of wild-type bacteria, but not of those lacking FbsC, increased their ability to bind to epithelial and endothelial cells.

During the preparation of this manuscript, a novel GBS adhesin named BsaB (bacterial surface adhesin of GBS) was described in GBS strain 515 (20). *In vitro* functional analysis of BsaB revealed that it participates in GBS binding to human fibronectin and laminin, in the adhesion of GBS to human epithelial cells, and in biofilm formation. Genome and sequence analysis revealed that FbsC and BsaB were identical and encoded by the same gene. However, whereas our results confirmed that

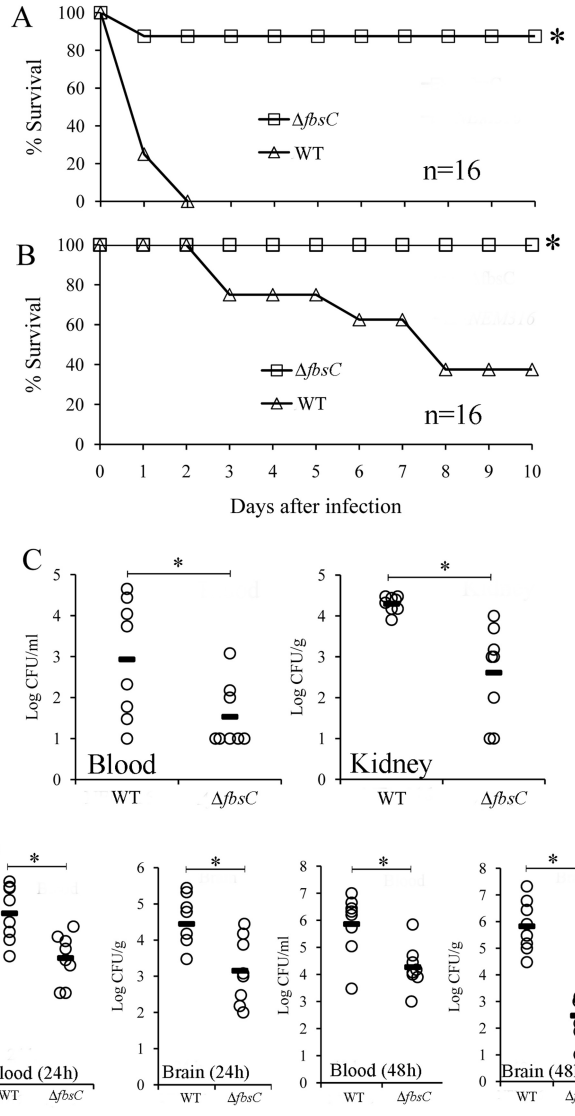

FIGURE 8. **FbsC is a virulence factor of GBS.** Comparison of lethality and bacterial burden in mice infected with wild-type NEM316 (*WT*) or its *fbsC* deletion (Δ*fbsC*) mutant. *A* and *B*, survival of CD1 mice following intraperitoneal infection with WT or with Δ*fbsC* strains using, as challenge, 4×10^8 (*A*) and 2×10^8 (*B*) cfu/mouse. *, $p < 0.05$ by log-rank Kaplan-Meyer analysis. *C*, blood and kidney colony counts at 24 h after intraperitoneal challenge with 1×10^8 cfu of WT or Δ*fbsC* strains. *, $p < 0.05$ by analysis of variance and Student's Neuman Keuls test. *D*, blood and brain colony counts in mice infected intravenously with 4×10^8 cfu of WT or Δ*fbsC* strains. *, $p < 0.05$ by analysis of variance and Student's Neuman Keuls test.

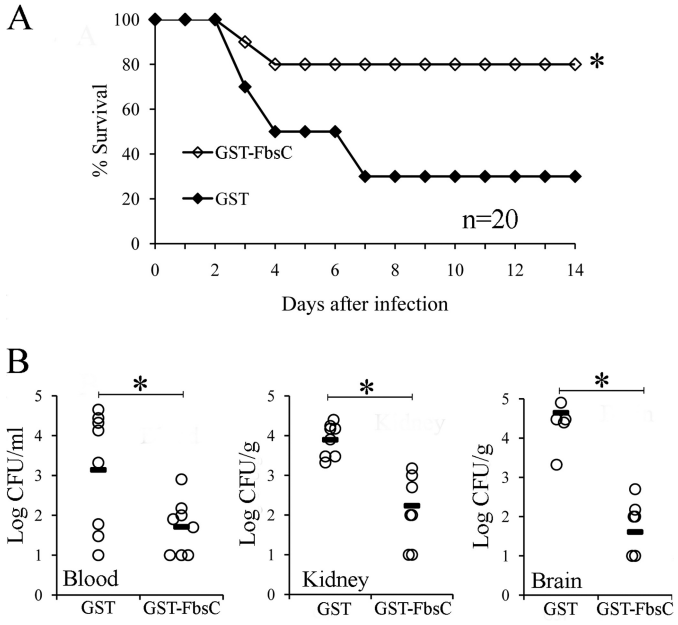

FIGURE 9. **Protective effects of GST-FbsC immunization against lethal GBS infection in mice.** *A*, animals were immunized with the recombinant GST-FbsC protein or with the GST control and challenged intraperitoneal with 2×10^8 cfu/mouse of wild type strain NEM316. *, $p < 0.05$ by log-rank Kaplan-Meyer analysis. *B*, organ cfu of mice immunized with the recombinant GST-FbsC protein or with the GST control and challenged intraperitoneally with 2×10^8 cfu. *, $p < 0.05$ by analysis of variance and Student's Neuman Keuls test.

FbsC/BsaB is involved in adhesion to epithelial cells and in biofilm formation, we demonstrate that this adhesin specifically binds fibrinogen, but not fibronectin. In fact, in their study (20), FbsC/BsaB binding to fibrinogen was not tested and the adherence rates to fibronectin were very low (less than 4%). We therefore maintained that FbsC/BsaB is a GBS surface protein that interacts with fibrinogen and conserved its designation as FbsC.

The respective contribution of cognate GBS fibrinogen-binding proteins (FbsA, FbsB, Srr11, Srr2, and now FbsC) for optimal binding to fibrinogen remains to be elucidated. However, it is likely that optimal adhesion of GBS to fibrinogen requires the simultaneous expression of different types of fibrinogen-binding proteins. It is interesting to note that, in this respect, FbsC preferentially interacts with the Bβ fibrinogen chain, as found here, whereas Srr1 and Srr2 both bind to the Aα chain (18, 19). The use of different adhesins may allow GBS to bind to different sites in the fibrinogen molecule, thereby promoting stronger interactions. Moreover, the relative importance of individual fibrinogen-binding proteins is likely to vary in different phylogenetic lineages, as suggested by the uneven distribution of each protein in these lineages (21). Initially, FbsA was proposed to be the major fibrinogen-binding protein in GBS (7, 15). Deletion of *fbsA* in the GBS 6313 (a strain related to NEM316 and belonging to the CC23 phylogenetic lineage) markedly impaired its fibrinogen-binding ability and host cells invasion. However, further studies investigating GBS strains belonging to the CC17 phylogenetic lineage have shown that the presence of the *fbsA* gene alone is not sufficient to produce robust adherence to fibrinogen and that FbsB plays a more important role in this process (16). More recently, Srr1 binding to fibrinogen was described as important for adherence to brain endothelium and the development of meningitis (19). However, Srr1 is absent in the CC17 phylogenetic lineage, which is associated with the vast majority of meningitis in newborns. Instead, CC17 hypervirulent strains express a specific serine-rich repeat protein called Srr2, which interacts with fibrinogen and promotes meningitis (18). The expression of FbsC in representatives of the CC1, CC19, and CC23, but not CC17, lineages is similar to that of Srr1. In CC17, the *fbsC* gene is present at the same locus but the protein is not expressed due to a lineage-specific frameshift mutation. Therefore, CC17 clones display an increased ability to adhere to fibrinogen with a repertoire of fibrinogen-binding proteins that includes FbsA, FbsB, and Srr2. This suggests that Srr2 plays a key role for fibrinogen binding in this lineage. A non-exclusive hypothesis may be the

differential expression of fibrinogen-binding proteins at the population level. Of note, FbsC and other fibrinogen-binding proteins are strongly regulated by the two-component system CovRS (also known as CsrRS), which plays an important role in controlling GBS adherence and biofilm formation (36). Importantly, whereas CovRS directly repressed FbsC expression (34), it also repressed that of the regulator Rga required for Srr1 expression (35, 51). Accordingly, inactivation of CovRS led to a dramatic increase in GBS binding to fibrinogen (52), but the contribution of individual fibrinogen-binding proteins remains to be determined in this genetic background.

In conclusion we have identified a novel fibrinogen-binding protein, designated as FbsC, which is required for cell adherence, biofilm formation, and invasion of epithelial and endothelial barriers by GBS. Further studies involving animal models of transmucosal infection (*e.g.* using respiratory or gastrointestinal challenge) will be needed to better assess the significance of FbsC-mediated adherence and invasion of epithelial barriers in the pathogenesis of GBS disease. In the present study, using an intraperitoneal challenge model, FbsC expression was essential for the induction of GBS disease and immunization with a recombinant form of the antigen largely prevented lethality. These observations suggest that FbsC may be a target, in conjunction with other antigens, for immune-based interventions to control GBS infections.

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